SOP 3: Counting Plants and Immature Monarchs

SOP 3 provides methods for measuring abundance of 1) milkweed, 2) nectar plants and 3) monarch eggs and 4) monarch larvae in the butterfly's migratory and reproductive ranges. These measurements will be used to quantify availability of food plants, and for evaluating habitat use, quality, and for estimating monarch survival rates. The procedures have been designed for measuring the four attributes in four different land cover/use types referred to as **sample draw strata**, including grasslands (unprotected and protected), right-of-ways, agricultural lands including lands in the Conservation Reserve Program (CRP), and urban/suburban areas (including parks, lots or gardens). For sampling purposes in the 2016 monitoring trial, these categories were subdivided or defined further into 5 sampling design strata and plot types: 1) protected grasslands (PRG plots), 2) unprotected grasslands (UPG plots), 3) roadsides (RDS plots), 4) agricultural fields with plots (AGC plots), and 5) agricultural lands enrolled in the CRP. Methods for sampling within a sixth stratum, urban/suburban areas (USA plots) will be developed in another trial conducted through the Chicago Field Museum.

The nectar plant monitoring procedures were adapted from the Prairie Reconstruction Initiative Retrospective Research protocol (Diane Larson, USGS) and the milkweed and monarch monitoring procedures were adapted from the Monarch Larva Monitoring Protocol (Monarch Larva Monitoring Project, 2016). These two elements were integrated to maximize efficiency when monitoring habitat and its use by monarchs.

These procedures require a moderate level of time and expertise, but provide a robust data set. For plant monitoring, a subdivided measuring frame is used to focus the plant species search. Because a small portion of the total area of interest is sampled, some species present at the site will not be captured by this method. Therefore, if a more complete list of nectar and milkweed species is desired from a site, a **meandering walk** can be added and performed mid-season when most species are mature enough to identify. A mid-season walk, however, will likely result in missing some early senescing (spring) or late developing (fall) species. Inclusion of the meandering walk during the 2016 monitoring trial is optional, based on the interests of the participating monitoring staff and time required to implement other monitoring tasks

Attributes Measured

- Time required to conduct this SOP
- Number of adult monarch butterflies (no./ ha)
- Behavior (category)
- Plant species used by adults for nectaring
- Temperature (ambient C^{o})
- Sky condition and precipitation category

Equipment and Supplies

- GPS or iPad with locating functionality
- Data sheets, clipboard, pencil or pen
- 100- meter tape (for checking distances and replacing missing flags)
- Compass

- Cord with key increments marked for quickly measuring short distances
- Pink wire flags and bamboo staffs (replacement materials)
- Black marker
- Plant identification materials (SM 3) and respective field guide
- Hand lens or magnifying glass
- Monarch identification materials (Appendix A) and butterfly field guide
- Camera
- Field thermometer

Data Recording

Record information on (see also SOP 5, SMs 4 and 5):

- Disturbances and survey layout coordinates using iPad survey form named SOP1_SiteDescription or paper data form labeled SOP 1 sheet 1
- Frequency of blooming nectar plants and counts of milkweed by species using iPad survey form named SOP3_BloomingNectarMilkweedCou… or paper form labeled SOP 3 sheets 1 and 2;
- Observations of adult monarch butterflies by behavior using iPad survey form named SOP2_AdultMonarchObservations or use paper data forms labeled SOP 2 sheet 3.

Sampling Frequency

Same plots, every 3 weeks.

Plot Area and Subsampling Layout

See SOP 1 for locating and establishing plots, and for conducting a rapid assessment (RAD) for milkweed plants at on-refuge sites before conducting the more intensive sampling and measurements described in this SOP. Plot set-up will vary according to the type of sampling strata and attributes being measured. Three of the sampling strata, Protected Grasslands (PRG), Unprotected Grasslands (UPG), and lands enrolled in the Conservation Reserve Program (CRP) plots will be sampled using a 300-m by 75-m plot. Roadsides (RDS) will be sampled using a series of 16 transects, 8 on a side that are each 75 m long. Intensive sampling of agricultural (AGC) plots will be conducted using transects and subplots placed between rows of crops or orchard trees. Use flagging and other markers to the extent allowed and remove after every visit if necessary, particularly for RDS and AGC plots. In all plots, plants and monarchs will be counted in (and sometimes between) 10 subplots on each of 16 transects, for a total of 160 subplots (150 target subplots plus 10 replacements). Each end of a transect should be marked with a white colored wire flag and Lat-Long coordinates recorded using a GPS (or iPad with GPS capability), or plotted UTM (NAD 83) coordinates.

- In grasslands (unprotected—**UPG**, protected—**PRG**, and agricultural lands enrolled in Conservation Reserve Program—**CRP**) plots, the 16, 75-m long transects are positioned 20 m apart and perpendicular to the long (300-m) side of the plot (Figure SOP-3.1).
- At roadside (**RDS**) plots, the 16, 75-m transects will be placed diagonally from the road edge (8 on both sides), out to a roadside width that is determined by an obvious change in vegetation type or 30 m (whichever comes first). Roadside widths must be a minimum of 6 m from the edge of the road (Figure SOP-3.2).

- In agricultural fields or orchards (**AGC)** plots that can be entered for intensive sampling, transects will be oriented in a furrow parallel to planted rows or trees (SOP 1) in a way that minimizes crossing of planted rows (Figure SOP-3.3). The number of transects per crop row will depend on the length of the rows with a maximum of 5 transects plus 5-m separation in a set (requires 400 m distance).
- Spacing among sets of transects will require 75-m and the number of rows equaling that distance will vary by type of crop and intervals between crop rows (Table SOP-1.3). When the rows are long enough (400 m long) use 5 transects in series with 5-m intervals between successive transects. In smaller fields sets will included fewer transects per row (Figure SOP 2.3), but will require more sets of transects with each set separated by 75 m.
- A preferred set up for maximizing amount of spatially overlapping data between plant and adult monarch abundance (i.e., maximizing correspondence of data collected under SOPs 2 and 3), will be 3 sets of 5 transects plus a single 16th transect placed within 4 different crop rows.
- Procedures on how to conduct SOP 3 in urban-suburban area (**USA**) plots will depend on the size of the area being measured. Guidance for these different situations are being developed through the Chicago Field Museum and University of Minnesota Monarch Lab.

Field Methods

Complete the following steps to implement this SOP:

1. Record a start time (24 hour clock) for SOP 3 tasks when leaving the vehicle with your gear, and head to the northwest corner of the target plot (avoid walking through the plot).
2. Using a GPS (preferred) or map with plotted coordinates and a compass, locate the northwest corner of the plot (marked with elevated pink flag and white-colored non-elevated flag inscribed as 'NW corner').
3. Lay out the 100 meter-tape between the paired, white colored transect flags which are located on the two 300 m sides of the plot boundaries. This will provide a straight transect line for you to walk. Note that transects 1 and 16 coincide with the two short sides (75 m) of the grassland plots. The 0 mark of the tape should be secured at the NW corner. Look for and record any disturbances occurring in 10% or more of the plot area.
4. Walk the first transect in the proper direction (toward the 75 meter mark on the tape, Figure SOP-3.2; placing the measuring frame at 10 subplots as you go. Subplots should be placed between the meter-marks indicated in Table SOP-3.1, which will shift according to subsequent visits to the plot. The distance between successive subplots should always be 7.83 m from the beginning of the preceding frame to the beginning of the next frame, except for the subplot 10 on each transect after the first sampling visit. Once frames are shifted 1 meter for subplots 1-9, the distance between subplot 9 and 10 becomes < 7.83 m (Table SOP-3.1) because subplot 10 is never shifted.
5. Record information about nectar plants, milkweed and monarch eggs and larvae as detailed below. Note: woody areas dense enough to prevent growth of understory herbaceous vegetation or that are too dense to walk through will not be measured; the 16th transect can be used if needed to make up subplots that are missed because of disqualifying conditions.

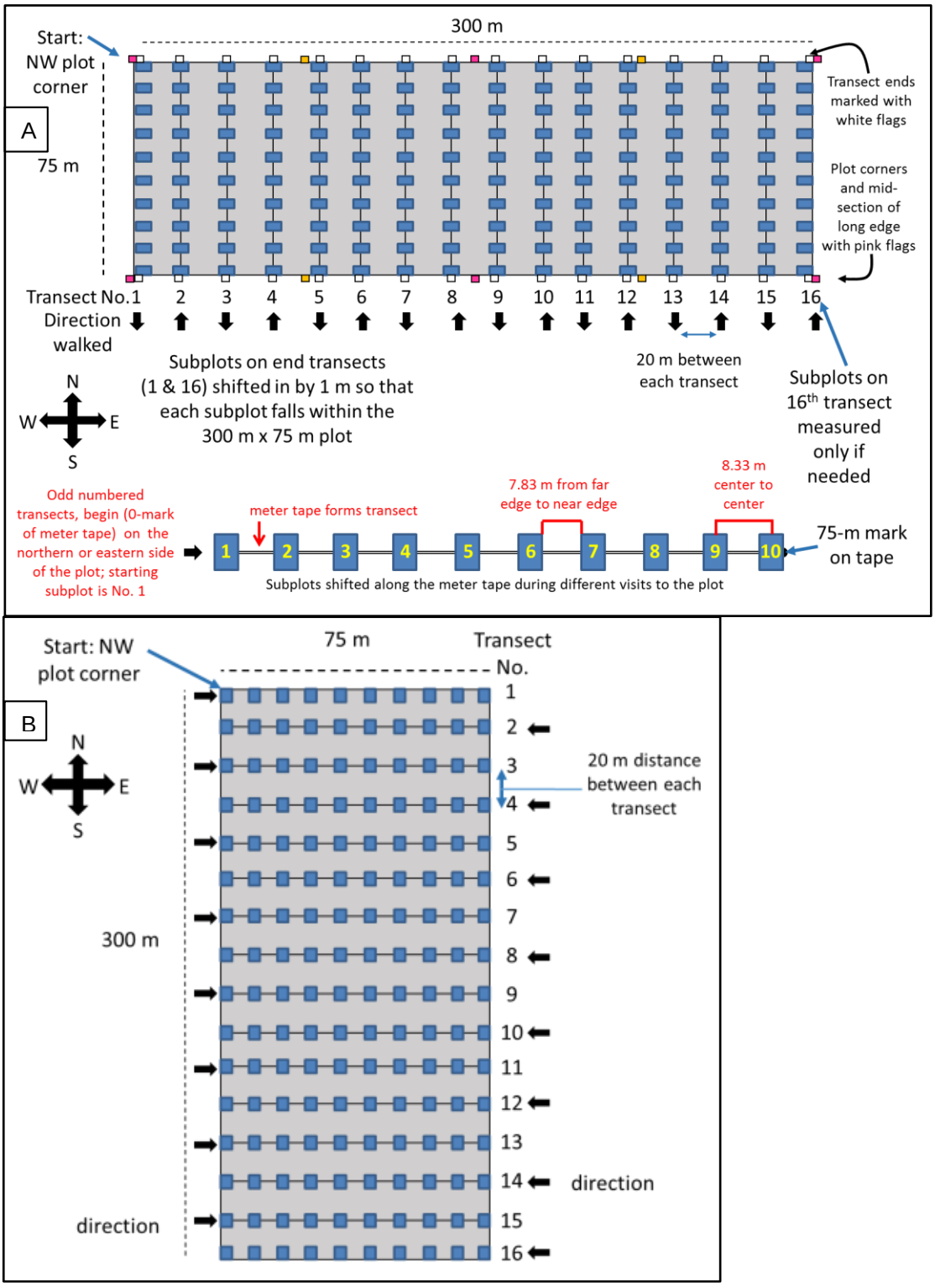

Figure SOP-3.1. Transects (black lines) and subplots (small dark blue rectangles) for open area plots oriented (A) West to East or (B) North to South. Each transect contains 10 evenly spaced subplots (0.5 m x 2 m). Note that subplots on the 1st and 16th transect are shifted into the plot by 0.5 m. Arrows indicate the direction of travel, starting points, and numbering of subplots on each transect.

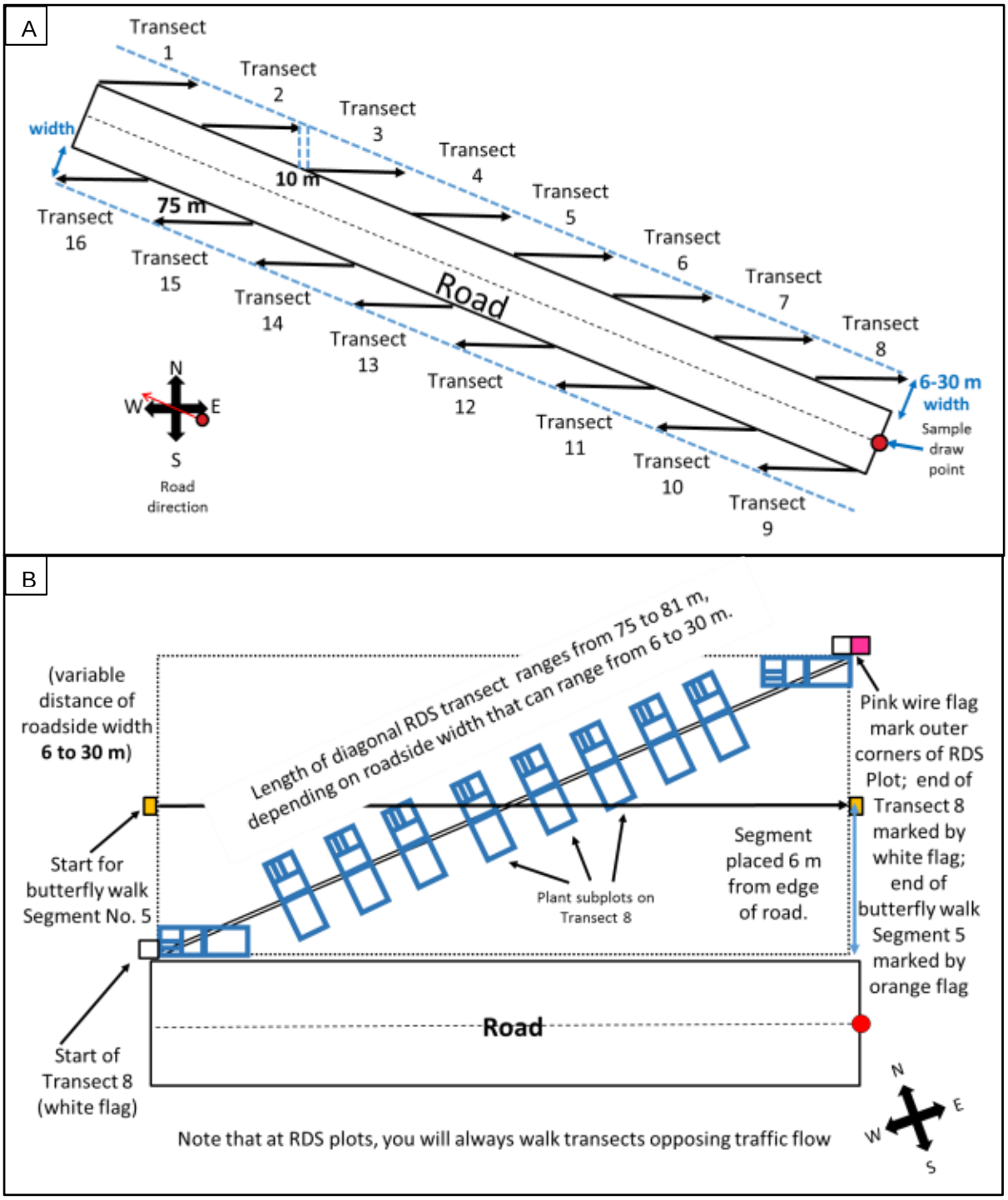

Figure SOP-3.2. Each roadside (RDS) plot is subsampled using a series of (A) 8, ~75-m transects placed diagonally away from a starting location at the road's edge on both sides of a road. Widths of area encompassing each transect range from a minimum of 6 m to a maximum of 30 m. Plants are measured in (B)10, 1-m^2 subplots positioned along each diagonal transect.

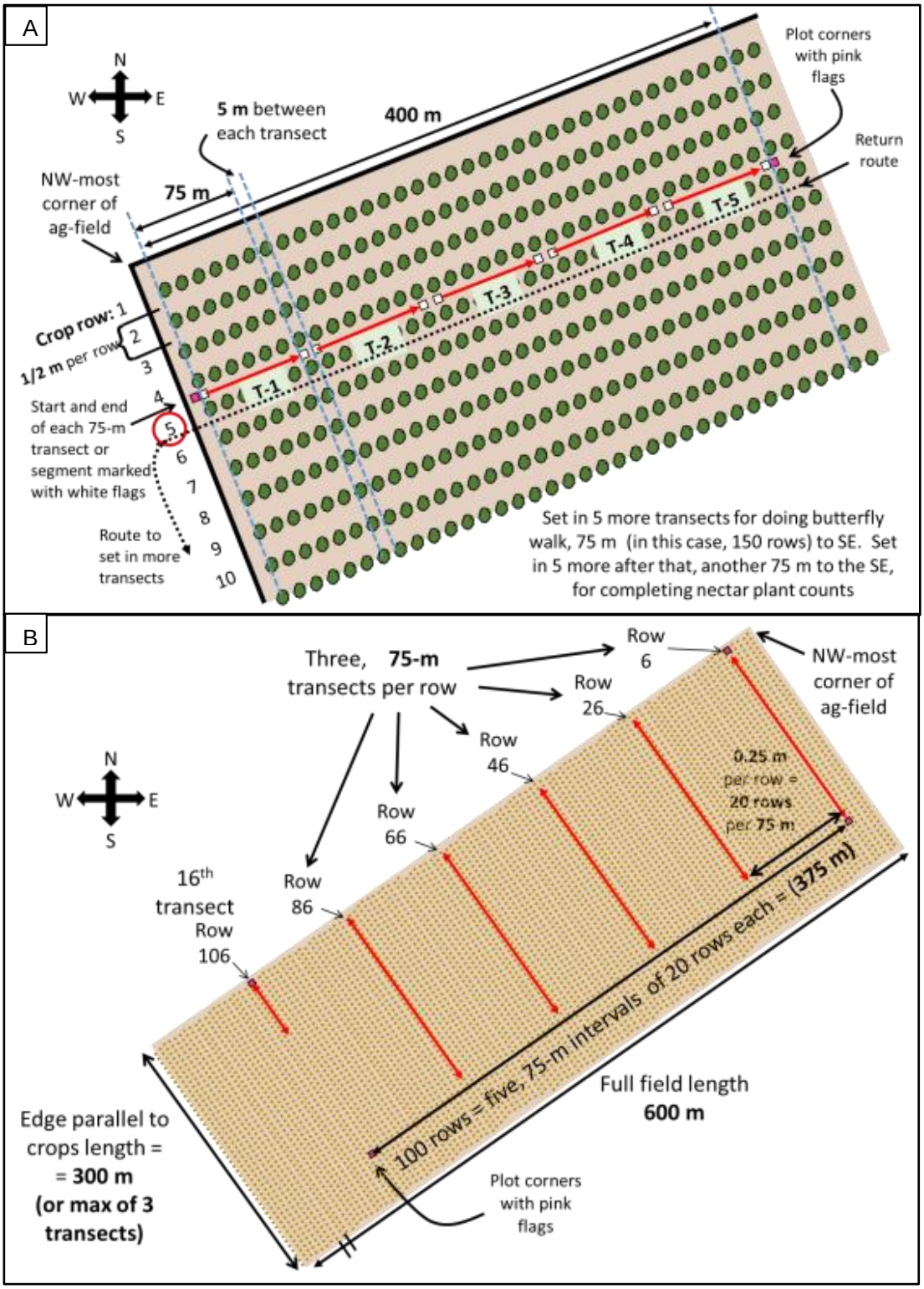

Figure SOP-3.3. Orientation, transect and subplot placement in AGC plots. The starting crop row is selected from a list of random numbers (see Table SOP-1.3). Transects and subplots are placed between crops with the long-side of each subplot oriented parallel to the meter tape. The number of transects placed within each crop row is determined by crop row length.

Measure Nectar Plant Abundance—Follow these steps to measure plant abundance using a **plant measuring frame** (Figure SOP-3.4) in each of 10 subplots on each of 15 transects (150 total).

1. Begin measuring and locating subplots according to the type of plot and transect layout.
    - In grassland plots, start from the northwest corner and place a measuring frame every 8.3 m along Transect 1, according to Table SOP-3.1 and Figures SOP-3.1 and 3.4 A and B.
    - In roadside plots with widths are long enough to accommodate frames set perpendicular to the transect line, place frames according to Table SOP-3.2 and Figure SOP-3.2B.
    - When roadside plots have shorter widths, place frames with the long edge parallel to the transect line using Table SOP-3.3 and Figure SOP-3.4C.
    - In agricultural fields with crops, place all frames as shown in SOP-3.4C using Table SOP-3.4.
    - Shift each frame starting edge 1 m forward on each subsequent visit in all plot types, except for leaving subplot 10 in place.
2. In each 1-m^2 subplot (1—10), record the frame-area code (1—5) for the smallest area in which each species of plant that has 1 or more flower(s) is observed (including milkweed [*Asclepias* spp.]). See SM 3 for the list of potential nectaring plants for each of the 3 refuges and vicinity, where the 2016 monitoring trial is being conducted.
    - Usually, at each subplot location you will place the measuring frame (or one end of it if the vegetation is tall) into the vegetation perpendicular to the transect, with the short side along and to the left of the transect line (Figure SOP-3.4. Adjust the vegetation so that only plants rooted within each subplot are recorded.
3. Repeat measuring subplots on each transect, noting where placement of the frame is different for outer transects in grassland plots or narrow widths at roadsides or in between crops.
    - The measuring frame is 0.5 m x 1.0 m rectangle that is divided into 4 nested areas of cumulatively larger size (Figure SOP-3.4). Cumulative area dimensions doubles with addition of each nested area
    - A fifth nested area results from flipping the measuring frame over yielding a total subsampling area of 1 m^2.
    - While placing subplots on the 2nd–15th transects, flip the measuring frame over the transect line for each subplot (Figure SOP-3.4A).
    - While placing subplots for the 1st and 16th transect, shift the transect 1 m into the plot so that the entire subplot area falls into the plot (Figure SOP-3.1A).
    - Placement of the frame in the first (No. 1) and last (No. 10) subplot on diagonal roadside transects, should hug the corners formed by a line along the road's edge (or parallel to roads edge) and a line out to the distance of the road-width, and where the transect begins or ends. (Figure SOP-3.2B).
    - For narrow roadside widths, subplots 2—9 on all transects, or all subplots on transects in agricultural plots with narrow spaces between crop rows (e.g., distances between crop rows < 1.0 m; Table SOP1.3), the measuring frame may need to be oriented so that the longer side is placed parallel to the transect line (Figure SOP-3.4B).

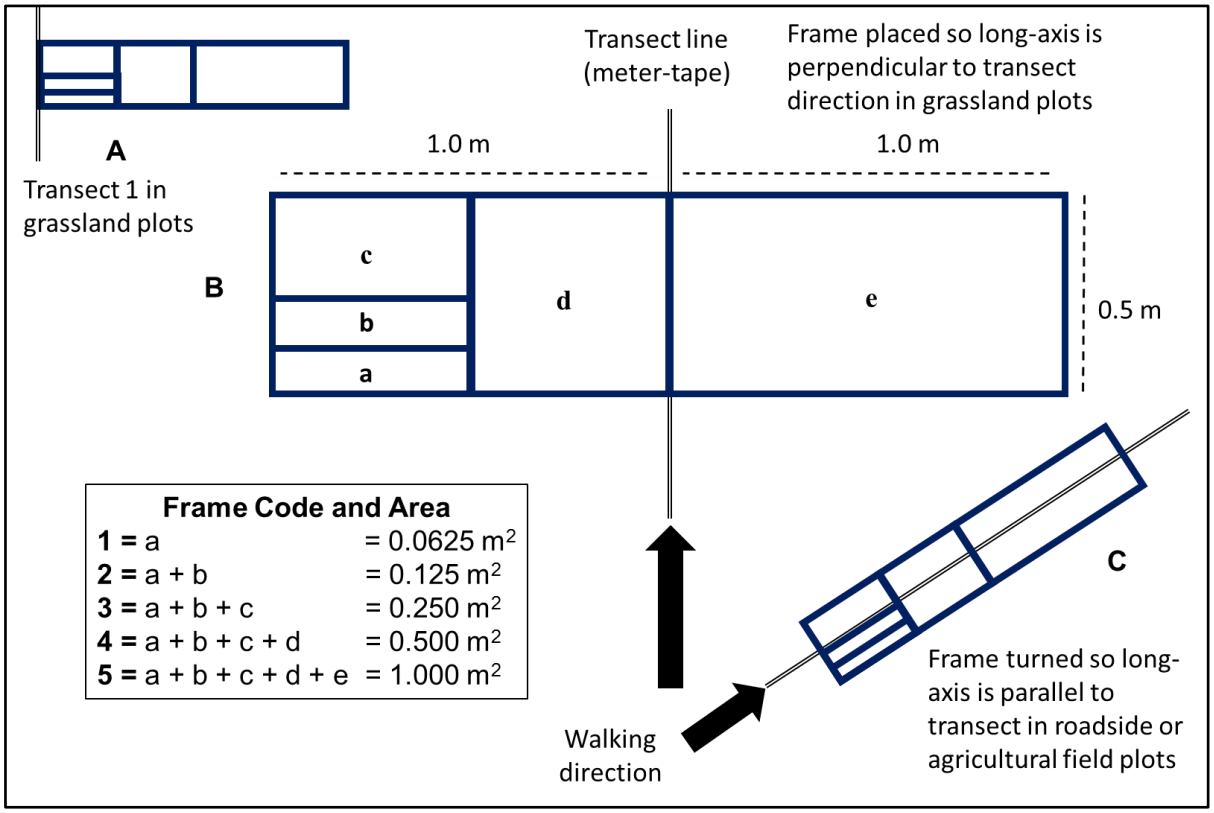

Figure SOP-3.4. Placement of the plant measuring frame (0.5 m x 1.0 m) and coding of nested-frame areas in subplots set along transects within (A) grassland type plots on Transect 1, or (B) Transects 2–15, or (C) at roadsides or agricultural plots where roadside widths or spacing among crop rows is too narrow to set the long edge perpendicular to the transect. The frame is used to identify the smallest area in which a nectar plant species in bloom occurs in a subplot.

Table SOP-3.1. Starting meter-tape marks for placement of the beginning edge of a 0.5 m by 1.0 m plant measuring frame on transects in grassland type plots (PRG, UPG, and CPR) for 6 sequential sampling visits (every 3 weeks). Frames are shifted 1-meter towards the end (subplot 10) each successive visit. Subplot No. 10 remands fixed for every visit.

Subplot No.	Sampling Visit No.1 (m)	Sampling Visit No.2 (m)	Sampling Visit No.3 (m)	Sampling Visit No.4 (m)	Sampling Visit No.5 (m)	Sampling Visit No.6 (m)
1	0.0	1.0	2.0	3.0	4.0	5.0
2	8.3	9.3	10.3	11.3	12.3	13.3
3	16.6	17.6	18.6	19.6	20.6	21.6
4	24.8	25.8	26.8	27.8	28.8	29.8
5	33.1	34.1	35.1	36.1	37.1	38.1
6	41.4	42.4	43.4	44.4	45.4	46.4
7	49.7	50.7	51.7	52.7	53.7	54.7
8	57.9	58.9	59.9	60.9	61.9	62.9
9	66.2	67.2	68.2	69.2	70.2	71.2
10	74.5	74.5	74.5	74.5	74.5	74.5

Table SOP-3.2. Starting meter-tape marks for placement of the beginning edge of a 0.5 m by 1.0 m plant measuring frame on transects at roadsides with varying widths (6 to 30 m) and transect lengths. Frames 1 and 10 are positioned as squared off to diagonal transect and frames for subplots 2—9 are set with long-edge perpendicular to transect (see Figure SOP-3.2B).

Plot width (m)	Meter Mark for Starting Edge of Plant Measuring Frame										
	SubP-1	SubP-2	SubP-3	SubP-4	SubP-5	SubP-6	SubP-7	SubP-8	SubP-9	SubP-10	Transect length (m)
6		8.3	16.6	24.9	33.2	41.5	49.8	58.1	66.4		75.24
7		8.3	16.6	24.9	33.3	41.6	49.9	58.2	66.5		75.33
8		8.3	16.7	25.0	33.3	41.6	50.0	58.3	66.6		75.43
9		8.3	16.7	25.0	33.4	41.7	50.0	58.4	66.7		75.54
10		8.4	16.7	25.1	33.4	41.8	50.1	58.5	66.8		75.66
11		8.4	16.7	25.1	33.5	41.8	50.2	58.6	66.9		75.80
12		8.4	16.8	25.2	33.5	41.9	50.3	58.7	67.1		75.95
13		8.4	16.8	25.2	33.6	42.0	50.4	58.8	67.2		76.12
14		8.4	16.8	25.3	33.7	42.1	50.5	59.0	67.4		76.30
15		8.4	16.9	25.3	33.8	42.2	50.7	59.1	67.5		76.49
16	0.0 m	8.5	16.9	25.4	33.9	42.3	50.8	59.3	67.7		76.69
17	Placed	8.5	17.0	25.5	34.0	42.4	50.9	59.4	67.9	Placed	76.90
18	'square'	8.5	17.0	25.5	34.1	42.6	51.1	59.6	68.1	'square'	77.13
19	with	8.5	17.1	25.6	34.2	42.7	51.2	59.8	68.3	with plot	77.37
20	plot	8.6	17.1	25.7	34.3	42.8	51.4	60.0	68.6	edges	77.62
21	edges	8.6	17.2	25.8	34.4	43.0	51.6	60.2	68.8		77.88
22		8.6	17.3	25.9	34.5	43.1	51.8	60.4	69.0		78.16
23		8.7	17.3	26.0	34.6	43.3	52.0	60.6	69.3		78.45
24		8.7	17.4	26.1	34.8	43.5	52.2	60.9	69.6		78.75
25		8.7	17.5	26.2	34.9	43.6	52.4	61.1	69.8		79.06
26		8.8	17.5	26.3	35.1	43.8	52.6	61.4	70.1		79.38
27		8.8	17.6	26.4	35.2	44.0	52.8	61.6	70.4		79.71
28		8.8	17.7	26.5	35.4	44.2	53.0	61.9	70.7		80.06
29		8.9	17.8	26.6	35.5	44.4	53.3	62.2	71.0		80.41
30		8.9	17.8	26.8	35.7	44.6	53.5	62.4	71.4		80.78

Table SOP-3.3. Starting meter-tape marks for placement of the beginning edge of a 0.5 m by 1.0 m plant measuring frame on transects at roadsides with varying widths (6 to 30 m) and transect lengths. Frames 1 and 10 are positioned as squared off to diagonal transect and frames for subplots 2—9 are set with long-edge parallel to transect (see Figure SOP-3.4C).

Plot width (m)	Meter Mark for Starting Edge of Plant Measuring Frame										
	SubP-1	SubP-2	SubP-3	SubP-4	SubP-5	SubP-6	SubP-7	SubP-8	SubP-9	SubP-10	Transect length (m)
6		6.6	13.3	19.9	26.6	33.2	39.8	46.5	53.1		75.24
7		6.6	13.3	19.9	26.6	33.2	39.9	46.5	53.2		75.33
8		6.7	13.3	20.0	26.6	33.3	40.0	46.6	53.3		75.43
9		6.7	13.3	20.0	26.7	33.4	40.0	46.7	53.4		75.54
10		6.7	13.4	20.1	26.7	33.4	40.1	46.8	53.5		75.66
11		6.7	13.4	20.1	26.8	33.5	40.2	46.9	53.6		75.80
12		6.7	13.4	20.2	26.9	33.6	40.3	47.0	53.7		75.95
13		6.7	13.5	20.2	26.9	33.7	40.4	47.1	53.9		76.12
14		6.8	13.5	20.3	27.0	33.8	40.5	47.3	54.0		76.30
15		6.8	13.6	20.3	27.1	33.9	40.7	47.4	54.2		76.49
16	0.0 m	6.8	13.6	20.4	27.2	34.0	40.8	47.6	54.4		76.69
17	Placed	6.8	13.6	20.5	27.3	34.1	40.9	47.8	54.6	Placed	76.90
18	“square	6.8	13.7	20.5	27.4	34.2	41.1	47.9	54.8	“square”	77.13
19	” with plot	6.9	13.7	20.6	27.5	34.4	41.2	48.1	55.0	with plot	77.37
20	corners.	6.9	13.8	20.7	27.6	34.5	41.4	48.3	55.2	corners.	77.62
21		6.9	13.9	20.8	27.7	34.7	41.6	48.5	55.5		77.88
22		7.0	13.9	20.9	27.8	34.8	41.8	48.7	55.7		78.16
23		7.0	14.0	21.0	28.0	35.0	42.0	49.0	56.0		78.45
24		7.0	14.1	21.1	28.1	35.1	42.2	49.2	56.2		78.75
25		7.1	14.1	21.2	28.2	35.3	42.4	49.4	56.5		79.06
26		7.1	14.2	21.3	28.4	35.5	42.6	49.7	56.8		79.38
27		7.1	14.3	21.4	28.5	35.7	42.8	49.9	57.1		79.71
28		7.2	14.3	21.5	28.7	35.9	43.0	50.2	57.4		80.06
29		7.2	14.4	21.6	28.8	36.1	43.3	50.5	57.7		80.41
30		7.3	14.5	21.8	29.0	36.3	43.5	50.8	58.0		80.78

Table SOP-3.4. Starting meter-tape marks for placement of the beginning edge of a 0.5 m by 1.0 m plant measuring frame on 75-m long transects in agricultural fields. Orientation is with the long-side of the frame placed parallel to the transect line as in Figure SOP-3.4C at every subplot.

SubP-1	SubP-2	SubP-3	SubP-4	SubP-5	SubP-6	SubP-7	SubP-8	SubP-9	SubP-10
0	6.6	13.2	19.8	26.4	33.1	39.7	46.3	52.9	59.5

4. There are 160 subplots (16 transects x 10 subplots/transect). Measure the first 150 qualified subplots you encounter. If a subplot contains incorrect strata or an impediment not detected by remote sensing (such as a stream or a recently tilled area), skip that subplot, but identify the impediment or inappropriate stratum or strata that were present. ***Only disqualify sections of the plot under extreme and obvious situations so as not introduce bias or potentially invalidate the plot for use***. In the cases where the intended and actual strata are clearly mismatched, use the following method for sampling.
    - If the first 150 subplots you encounter all contain correct strata, you do not need to walk the plot's 16th transect.
    - If you disqualified subplots in the plot's first 15 transects, then measure that many extra subplots on the 16th transect. For example, if you disqualify 2 subplots on the first 15 transects, measure the first two subplots in the 16th transect.
    - If you disqualify more than 10 subplots in the first 15 subplots, monitor all 10 subplots (if qualified) in the 16th transect. In this case, you will measure fewer than 150 subplots for the entire plot.
5. At each subplot, systematically look for blooming plants in the cumulatively larger areas of the frame labeled 1 through 5, and record data about relative frequency of nectar plants.
    - Starting with the smallest area in the measurement frame (Figure SOP-3.3B: **subdivision a**, record species with blooming flowers on the nectar plant datasheet (SOP 3 sheet 1 or iPad survey form SOP3_BloomingNectarMilkweedCou…).
    - **Use a separate data sheet for each transect**, filling out one column for each of the 10 subplots.
    - Only record each species in the smallest area in which it occurs, as follows: in the appropriate column for the given subplot, record a "1" for all species seen in subdivision a of the measuring frame. Record a "2" for any new species seen in the cumulative area of subdivisions a and b. Continue similarly for cumulative areas coded 3 and 4. Then flip the measuring frame lengthwise for completing the cumulative subplot area (0.5 x 2.0 = 1 m^2) and coding any new flowering nectar plant species as 5 (Figure SOP-3.4).
    - When recording blooming plant species, use the first three letters of genus and first three letters of the scientific name for a plant species given in the USDA Plant Database (SM 3).
    - Note that in some cases, more than one species will have the same code (e.g., ASCVIR could be either *Asclepias viridis* or *A. viridiflora*), so be particularly careful with such species (SM 3 and Appendix D).
    - If you encounter a blooming nectar plant you cannot identify, follow steps listed in Appendix C for naming the unidentified plant on the data sheets (or iPad) so that once identified, the data sheet can be rectified.

Measure Milkweed Abundance—For each 0.5 x 2 m subplot, record the number of milkweed plants (and stems if relevant) by species (SOP 3 data sheet 2 or iPad SOP3_BloomingNectarMilkweedCou).

1. Use the following rules and definitions to record milkweed abundance:
    - Only record information about stems rooted within the 2 m x 0.5 m subplot, even if the plant includes stems out of the plot.

- If a milkweed plant is blooming, record it on SOP 3 data sheet 1, and again on data sheet 2; you are treating the nectar and host plant functions of milkweed separately.
- A **milkweed plant** is defined as all above-ground stems of milkweed originating from a visually-identifiable, common central point in the ground.
- A single milkweed plant may be composed of one or multiple **stems** depending on the species. For example, *A. viridis* (green antelopehorn milkweed) plants may grow multiple stems per plant (Figure SOP-3.4).
- Other frequently encountered species of milkweed that often grow multiple stems per plant are *A. tuberosa* (butterfly weed) and *A. incarnata* (swamp milkweed). If you encounter these plants, count each cluster of stems originating from the same central point as a single plant. Note that stems from the same plant may be separated by soil.
- Record data about both plant number and stem per plant in the appropriate columns of SOP 3 sheet 2. Stems of some species of milkweed may branch aboveground, but branches originating from a single stalk should be counted as one stem.
- *A. syriaca* (common milkweed) is an exception to the general rule outlined in the previous paragraph. A single *A. syriaca* plant may grow many **ramets** (above-ground as stalks) that are separated by small or large distances, but without excavating roots, it is impossible to tell if ramets are from the same or different plants (Figure SOP-3.6).
- Record each individual stalk of *A. syriaca* as a plant in the "Plant/Ramet" column of SOP 3 sheet 2. Multiple stalks from a single point (with no soil separating stalks), however, should be recorded as a single plant with multiple stems (importantly, it is rare for *A. syriaca* to have multiple stems originating from the same point).

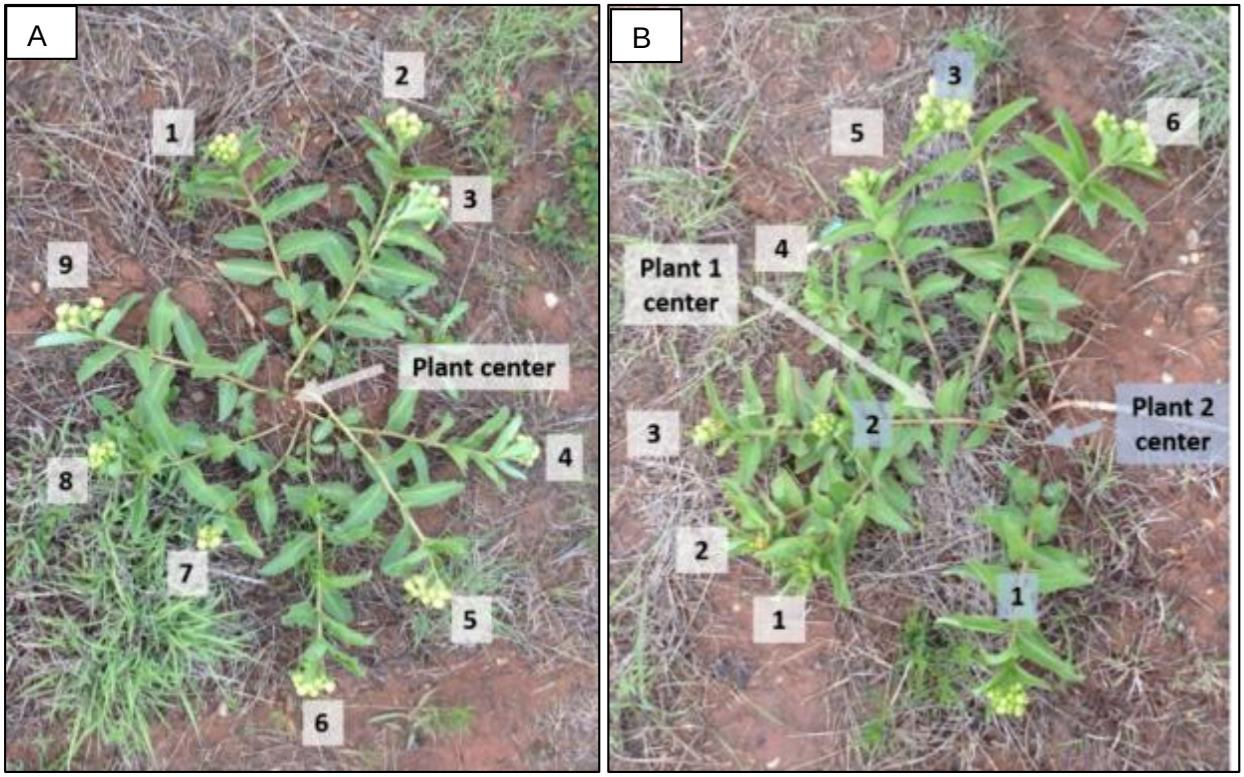

Figure SOP-3.5. *A. viridis* (green antelopehorn milkweed) plants and stems. (A) One *A. viridis* plant with 9 stems. Stems originate from a central point and are separated by soil. (B) Two *A. viridis* plants. One plant has 6 stems and the other plant has 3 stems. (Photos courtesy of Kristen Baum).

Figure SOP-3.6. *A. syriaca* (common milkweed) plants. Above-ground stalks (ramets) could be from different or the same plant. Count each individual stalk as an individual plant unless there is no soil separating stalks (Photo courtesy of Denise Gibbs).

Measure Monarch Egg and Larva Density—Search the milkweed plants in the subplots for immature monarchs (eggs and larva). To increase the sample size of milkweeds searched, you will also search plants along a ~2-m swath that you encounter as you walk between subplots. Recording all of these things will seem complicated at first, so make sure you understand this process. Keep in mind that the milkweeds in the subplots are being used for two things: milkweed density and per plant monarch density. Milkweeds between the subplots are only being used for per plant monarch density, so measurements to the exact inch are not important as you search the 2-m swath. Milkweed (blooming or nonblooming) will only be recorded on SOP 3 sheet 2. See examples at the end of this SOP.

1. Search each milkweed that you encounter in the 0.5 m x 2 m subplot for monarch eggs and larvae.

- Record the number of eggs and larvae (identified to instar) and the corresponding plot on SOP sheet 2.
- To increase the number of milkweed plants that you search, search each milkweed stem encountered in a swath approximately 2 m wide as you walk between plots.
- Record the number of milkweed plants (and stems, if appropriate) by species, and the number of eggs and larvae that you see on these stems.
- Record location information for plants found outside of the subplots by noting the two plot numbers you walk between (subplot ***s*** –subplot ***s + 1***). For example, any milkweeds checked between subplots 1 and 2 on a transect get labeled as 1–2; between subplots 4 and 5 as 4-5, etc.
- Always record the number of milkweed plants (and stems) that you examine. This includes milkweeds on which you do not observe monarchs.
- The result will be an estimate of monarch density at the site, calculated as a proportion of milkweed plants with monarchs.

2. Remember that monarch eggs and larvae can be hard to find!
    - To examine a milkweed plant, look carefully at all parts of the plant, including the bottoms of the leaves, the area within the small leaves at the top of the plant, and buds and flowers if they are present.
    - Keep an eye out for caterpillar clues, such as chew marks on the leaves.
    - Handle the plants carefully to avoid knocking any larvae off of them.
    - Use the pictures of each instar at the end of this SOP (or in Appendices A and B) to help you distinguish monarchs from other insects, and the five instars.
3. Only record data for milkweed plants in the ~2 m swath.
    - If you see a monarch egg or larvae on a plant outside of this swath, don't count it.
    - Looking at a plant just because you saw a monarch on it is not a random observation, and will skew the data you collect.
4. If you encounter a dense patch of milkweed (for example, A. verticillata or whorled milkweed grows in dense patches) between subplots, it might be impractical to count and search each stem.
    - In this case, count and search the stems in a 1-m (or even 0.5-m if very dense) swath instead of a 2 m swath.
    - Record only the number of stems you search and the number of monarchs you find. Again, if you have decided that you're only going to monitor a 1-m swatch, and you see a monarch on a plant outside of that swatch, you should not count it.
    - Alternatively, if you encounter a dense patch of milkweed between subplots, pick a number and monitor every 3rd, 4th, or 5th, etc stem for milkweed.
    - Be consistent and use the same number for the entire patch. Do not selectively choose to search certain stems as this is not random and will skew the data you collect.

Manage Collected Data—Enter, check, and store data following instructions in SOP 5. Data recorded on paper forms should be checked at the end of each day and entered as soon as possible, but at least once a week, into the appropriate spreadsheet.

Adult Monarchs—Male and female monarchs can be distinguished easily. Males have a black spot on a vein on each hind wing that is not present on the female. The ends of the abdomens are also shaped differently in males and females, and females often look darker than males and have wider veins on their wings.

Male Monarch Butterfly
(photo courtesty of Michelle Solensky)

Female Monarch Butterfly
(photo courtesy of Barbara Powers)

Eggs and Caterpillars—Early life phases of the monarch butterfly occur as eggs and larvae (caterpillars) as up to 5 instar stages of development. Eggs and caterpillars are found on species of milkweed plants (Asclepias spp.).

Monarch egg on milkweed leaf — The egg is a little more than 1 millimeter tall. *(Photo courtesy of Lynda Andrews)*

Close-up of monarch egg — Note the pointed shape, the glossy color, and the vertical striping. *(Photo courtesy of Michelle Solensky)*

Monarch egg (left) and latex drop (right)
(Photo courtesy of Anurag Agrawal)

Live monarch egg about to hatch
(Photo courtesy of Valerie Evanson)

Dead monarch egg – Note the "puddle" of dead larva in the bottom of the egg.
(Photo courtesy of Valerie Evanson)

Monarch first instar consuming eggshell — Note the dull greenish-grey color, and the size (not much bigger than the egg).
(Photo courtesy of Mary Holland)

First instar feeding damage — This circular feeding pattern is an indication that a monarch first instar was on the plant at some point.
(Photo courtesy of Tom Collins)

Monarch second instar — Second instar larvae have a distinct pattern of black, white, and yellow band, and the body no longer appears transparent and shiny. *(Photo courtesy of Monarch Lab)*

Monarch third instar — This third instar monarch has just molted. As monarch larvae develop, they increase in size and their stripes become more distinct. Third instar larvae usually feed using a unique cutting motion on leaf edges. *(Photo courtesy of Monarch Lab)*

Monarch fourth instar — Fourth instar monarchs front tentacles extend beyond the tip of the head. Internal changes, including the development of reproductive structure, begins to occur in late instar monarchs. *(Photo courtesy of Monarch Lab)*

Monarch fifth instar — Older monarch larvae have bright yellow, black and white striping and 2 pairs of tentacles (on front and back ends). *(Photo courtesy of Richard Hicks)*

Monarch instars — The entire larval stage in monarchs lasts from 9-14 days under normal summer temperatures. The speed of monarch development is temperature dependent. *(Photo courtesy of Monarch Lab)*